PARAMECIUM INFUSION HISTORIES

I. Hydrogen Ion Changes in Hay and Hay-Flour Infusions

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The data presented in the following article were collected in the course of observations made upon a series of forty-seven infusions, in an effort to accumulate as complete a record as possible of the events which take place in a culture of *Paramecium multimicronucleatum*. This article will be restricted to pH behavior. Other phases of the culture history will be dealt with in articles to follow.

Historical

The literature of this subject is quite limited.

Bodine (1921), using colorimetric methods, studied the hydrogen ion behavior of various infusions in which he was able to raise mixed protozoan cultures. His infusions passed through acid and alkaline cycles in a manner which closely resembled the behavior of the infusions that we are reporting as types, with the difference that the various cycles were retarded in his infusions. This was probably due to the larger volumes of the infusions which he studied. Our large cultures displayed a similar retardation.

Pruthi (1927) studied the hydrogen ion concentration of a series of hay infusions of different sizes which were made without boiling, and which were allowed to develop protozoan populations without seeding. In these studies he recorded changes of pH which were quite similar to those which are reported in this paper.

Darby (1929 and 1930) has demonstrated in a very convincing manner that the division rate of *Paramecium caudatum* and *P. aurelia* is greatest at a pH slightly less than 7.00, and that an increase or decrease of hydrogen ion concentration from this optimum will reduce the division rate.

Beers (1927) has reported the hydrogen ion concentrations of cultures of bacteria alone, and of bacteria and *Paramecia*. He says, "It is seen that the hydrogen-ion concentration of fresh infusion was pH 6.2, and that it increased to 5.8 during the first twenty-four hours, after which it steadily decreased as the infusions grew older. This

succession of changes in hydrogen-ion concentration was observed regularly in all infusions prepared." It will be noted that his results agree only in a very general way with ours, both with respect to the pH variation and the time of greatest hydrogen ion concentration.

Phillips (1922), experimenting on the feeding of known kinds of bacteria to *Paramecium*, reported at the termination of her work that two of her cultures showed a pH of 8.2, while the third had a pH of 8.4.

Johnson (1929) reported that the cultures from which he took *Paramecium* ranged between pH 7.4 and 8.0. He described this stage as one in which the *Paramecia* were scattered throughout the culture.

Beers (1927), while investigating the encystment of *Didinium* in buffered solutions of varying hydrogen ion concentration, found that the limits within which the organism could live were approximately pH 5.0 to pH 9.6. Crane (1921) found practically the same limits for *Paramecium*, and Johnson (1929) determined that *Paramecia*, in drops of solution tested colorimetrically, were killed at pH 5.0. His electrometric readings indicated a pH somewhat lower.

Materials and Methods

This series of studies was made on infusions of three sizes. The medium size, 700 cubic centimeters, was considered the standard, and the major portion of the cultures contained that amount of liquid when made. Three infusions of one third that size were studied, as were three of 7 litres. The 700 cc. cultures were all kept in containers of uniform size and shape. In the discussion which follows, only the 700 cc. cultures will be considered, unless the larger or smaller infusions are mentioned specifically.

Of the infusions studied, some were loosely covered, others were open. Experience proved that the loosely covered jars lost two cubic centimeters by evaporation daily, while the open jars lost six times that amount. This uniformity of evaporation rate was obtained by keeping all of the cultures in a constant temperature room at 27° C.

The standard culture of 700 cc. capacity became divided into a considerable number of groups, dependent upon the quantities of hay and flour that were used in preparing them. The practice was to prepare at least three infusions of any one of the more important types simultaneously, and the facts so obtained were again checked in some cases by preparing a single infusion of the same kind later. The composition of these infusions is most readily described through the agency of the table which follows.

TABLE I

Infusions in which Paramecium Lived and Multiplied

No. Infusions Made	Grams of Hay Used	Grams of Flour Used	Covered or Open
9	1	0.1	Covered
6	1	0.	"
1	2	0.	"
1	4	0.	"
4	1	0.1	Open
4	1	0.	"

Infusions in which Paramecium did not Live

No. Infusions Made	Grams of Hay Used	Grams of Flour Used	Covered or Open
4	4	0.4	Covered
1	4	0.1	"
1	2	0.2	"
2	8	0.8	"

All infusions were prepared by bringing distilled water to a boil in a covered granite kettle, adding the required amount of timothy hay, and boiling for ten minutes. The uniformity of the hay for the entire experiment was controlled by passing a considerable amount of the original hay through a meat chopper, and then thoroughly mixing the cut product. White wheat flour, when needed, was mixed into the hay and added to the infusion with it.

Infusions so prepared were poured into their respective containers and allowed to cool until the following day, when they were seeded with two hundred *Paramecium multimicronucleatum* of a pure line. This formula is essentially similar to the one used by Packard which was on file in this laboratory.

Hydrogen ion concentration measurements were made at frequent intervals during the life of the culture, as were population counts. These data were plotted against time in a series of graphs. This method proved convenient, as it showed clearly the relationships existing between population per cc., volume of infusion remaining, and hydrogen ion concentration on any day of the infusion life.

The pH readings were made with a No. 5270 Youden Apparatus.

All infusions were stirred violently at intervals of one to three days, when population counts were made.

Observations

From the data gathered, certain facts concerning the hydrogen ion concentration of a hay, or of a hay-flour culture of *Paramecium multimicronucleatum* stand out with such clearness, and are so typical of the results obtained in this experiment, that it seems safe to illustrate the general hydrogen ion history of a successful culture by contrasting one culture which failed, probably due to the prolonging of the acid period, with a second one which succeeded. We presume that this success was due to a more favorable pH behavior on the part of the second culture. Culture 36, of which Plate 1 is the graphical record, made with one gram of hay and 0.1 gram of white flour, developed a culture in a normal and satisfactory manner. It had a pH of 6.93 within an hour of the time that it was made, and while it was still slightly warm. During the next twenty-four hours, it went down to pH 6.08, at which time it was seeded with two hundred *Paramecia*. For some reason it seemed to hesitate in its pH reducing process. This is the usual behavior. The second day found it still pH 6.09 at 10 A.M., but the evening of the same day showed a pH of 5.64. After midnight of the third day, when the pH was 5.15, it reached its turning point, for by 11 A.M. of the fourth day the reading was 5.22. Midnight of the fourth day showed pH 5.38, and from this point it made a rapid return to pH 6.95, the hydrogen ion concentration which it reached by midnight of the eighth day.

The hydrogen ion behavior of this culture is, essentially, the behavior of each culture in the series of experiments in which the *Paramecia* survived the extremely acid period. In most of the cultures the highest hydrogen ion concentration noted was between pH 5.00 and 5.50, although culture 35 developed a pH of 4.83 without killing all of its 200 seed *Paramecia*. Such an acid condition was usually developed in about four days, and a condition of approximate neutrality was reattained in from eight to ten days. The following ten days characteristically fluctuated between slight acidity and slight alkalinity, following which there was a gradually increasing alkalinity throughout the remainder of the life of the infusion. This final alkalinity seldom was more than pH 7.5 when 250 cc. of the infusion remained, and usually it was less.

In the open cultures, where a greater reduction of the volume took place, a rising pH resulted. Culture 4, the graph of which is not shown, recorded a pH of 8.31 when only 20 cc. of the original 700 remained. Infusions such as No. 4, which had lost a greater portion of the original fluid through evaporation, usually contained 2000 or more *Paramecia* in each cc. of the culture. Such remarkable concentrations of the

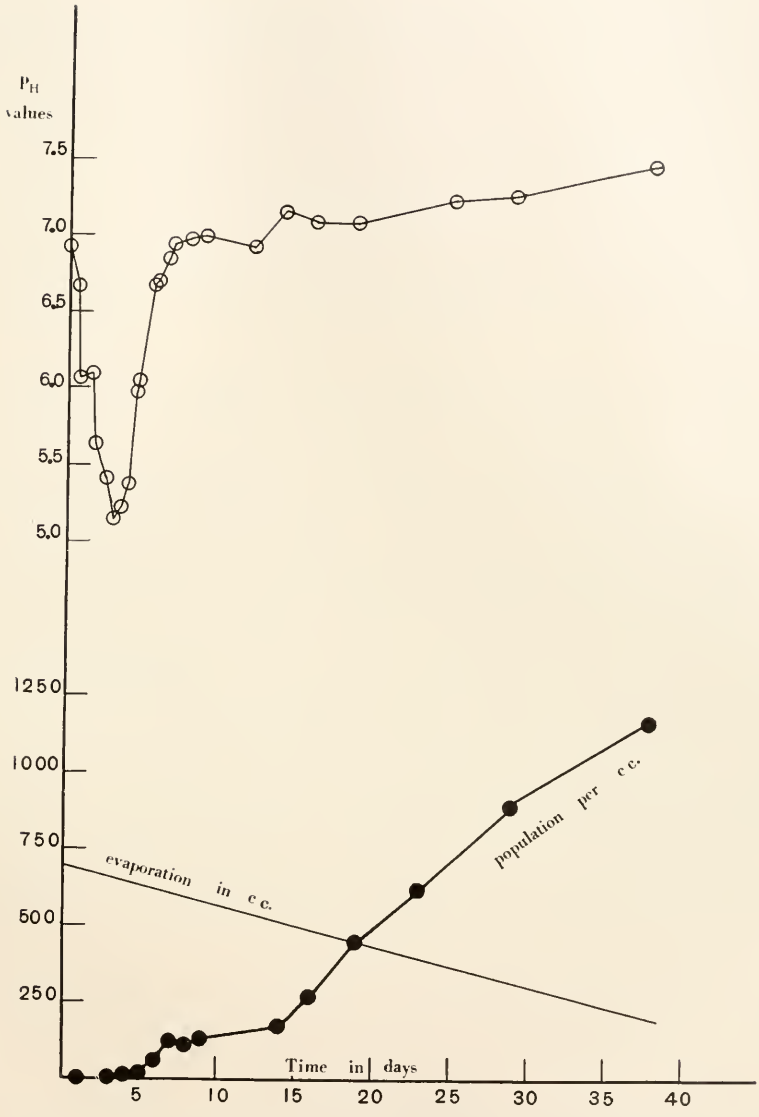

PLATE 1. This graph shows the typical hydrogen ion behaviour of a hay-flour infusion which was seeded with 200 *Paramecium multimicronucleatum* before it had reached its maximum acidity.

This pH record is typical of all cultures in this series of experiments, in which the *Paramecia* lived.

Paramecium population might be continued for ten days or more, if open jars, which had evaporated the greater part of their infusion, were covered.

It was repeatedly noticed that the *Paramecium* population in a successful culture was concentrated near the top in a younger infusion; was uniformly distributed throughout the container in a culture of medium age; and finally, that the animals concentrated at the bottom of the culture. It seems probable that this systematic behavior is associated with the changing pH conditions that are reported in this paper. Johnson (1929), Child and Deviney (1926), and Pruthi (1927) have reported somewhat similar observations of population distribution.

Such a distribution of animals did not appear to be caused by differences in hydrogen ion concentration at different levels of the infusion. The electrometric readings of the pH of material taken from the top, middle, and bottom of a container were alike when investigated on different occasions. Color indicators added to cultures showed no hydrogen ion concentration differences at the various levels.

Plate 2 is the graphic record of Culture 34. It serves as a contrast to the cultures which were successful. The medium was made by boiling 4 grams of hay and 0.1 gram of flour in the usual amount of water. As is shown in the attached graph, this culture also started at neutrality, hesitated in its pH reducing process when the same quantities of *Paramecia* and infusion were added, after which its pH continued to reduce until it had reached 5.05 by midnight of the third day. If the hydrogen ion concentration had returned to normality in this infusion as it did in Culture 36 which was previously described, it seems probable that a successful culture would have been established. Instead, the pH readings remained between 5.00 and 4.90 for the next five days, at which time an examination showed that all of the animals were dead. The hydrogen ion concentration of this infusion did return to normal a few days later. It was then seeded with a second quota of 200 *Paramecia* and these animals multiplied satisfactorily. The pH when this infusion was seeded the second time was 7.3.

In all such infusions, the list of which is given, it appeared that a prolonged acid phase was induced in the early stages of the infusion by the addition of either too much hay or too much flour. When no flour was used, as much as 4 grams of hay did not induce either superacidity, or acidity too prolonged. The addition of 0.1 gram of flour to such a formula produced a medium which killed. If one tenth of one gram of flour was used, the hay had to be reduced to less than two grams if the seed *Paramecia* were to live.

Such results would have caused us to altogether discontinue the use of flour in the making of the media, had we not noted that the densest populations were secured when flour was used. The choice formula, therefore, came to be one gram of hay and one tenth of one

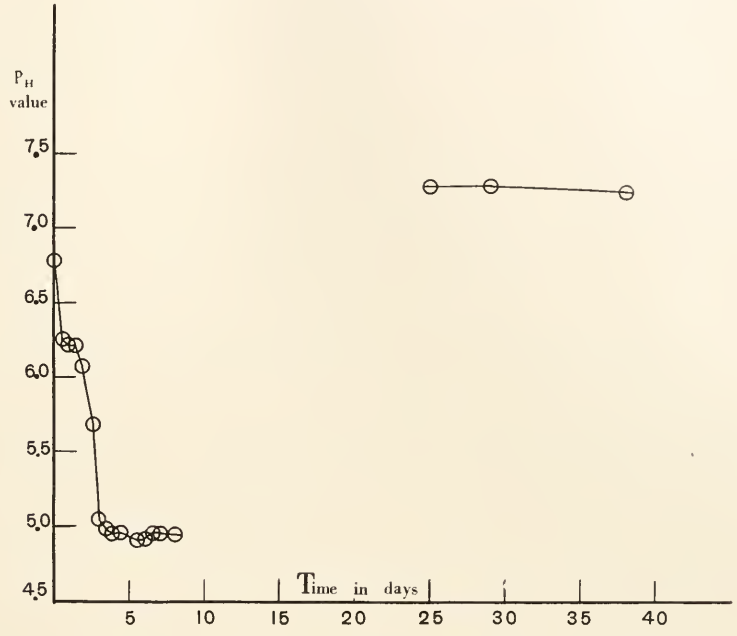

PLATE 2. This graph shows the typical pH behavior of a hay-flour infusion which killed its 'seed' population of 200 *Paramecium multimicronucleatum*, presumably as a result of the prolonged superacidity.

No hydrogen ion concentration readings were made between the eighth and the twenty-fifth days. 'Seed' *Paramecia* introduced on the twenty-fifth day grew successfully at the hydrogen ion concentrations shown.

gram of flour in 700 cc. of distilled water. Such a culture in a covered jar would usually support from 300 to 700 animals for each cubic centimeter of the infusion, producing a total population of 200,000 to 500,000.

DISCUSSION

A consideration of the data which have been presented indicates that the length of time in which the extremely acid phase continues, determines whether the infusion will kill the seed *Paramecia* which are introduced the second day. This acid phase is accompanied by fermentation, and is evidently brought about by bacterial activity. The degree of hydrogen ion concentration is probably a factor in determining whether all of the seed *Paramecia* will be killed. Crane

(1921) states that pH 5.0 is the greatest hydrogen ion concentration in which *Paramecium* can live for twenty-four hours. Culture 35 of this series developed a pH of 4.83 for a few hours without killing the *Paramecia* in it, but our usual culture did not have a hydrogen ion concentration greater than pH 5.0.

The second stage of the pH behavior, in which the infusion returns to normality and then becomes alkaline, is probably brought about by a second cycle of bacteria. Peters suggested the probability of such bacterial cycles as early as 1907.

Our observation of similar hydrogen ion concentrations at the top, middle, and bottom of cultures would seem to disagree with those of Peters (1907), who found an increase in the titratable acidity as materials were drawn from deeper levels. Fine's (1912) series *A* and *C*, from which he did not strain out the hay, paralleled the behavior of Peters' infusions; but his series *B*, from which he had removed the hay, showed practically no differences in titratable acidity at different levels. Fine believed that this acidity was caused by bacterial action, and he suggested that the greater acidity at or near the bottom was due to the concentration of the bacteria about the hay. Such an explanation might account for the uniformity of our pH readings at different levels, for our cultures were violently stirred at intervals of one to three days, when population counts were made. Such treatment would scatter the bacteria throughout the culture.

We plan to try to determine the species of bacteria which cause these changes; the stages of the infusion in which each bacterium is most numerous; the pH condition which the metabolism of each bacterium induces; and the maximum, minimum, and optimum pH for each. Hargitt and Fray (1917) and Phillips have made a very creditable beginning on bacterial identification and description.

Summary

A study of forty-seven *Paramecium multimicronucleatum* cultures made by boiling varying combinations of timothy hay and wheat flour in distilled water and seeding with 200 pure line *Paramecia* on the second day has yielded the following data:

(*a*) Cultures experienced a changing pH cycle which was invariably quite similar to that shown in Plate 1, if the *Paramecia* lived.

(*b*) The most successful timothy hay-flour medium was made by boiling one gram of hay and one tenth of one gram of flour in 700 cc. of water for ten minutes.

(*c*) *Paramecia* were observed to live in culture media whose pH ranged from 4.83 to 8.31 after the solutions had been violently stirred.

(*d*) As cultures evaporated, the alkalinity increased after the first four days. A hydrogen ion concentration of 8.31 was observed in infusion 4 when 20 cc. remained of the original 700. This infusion had a population of 6000 *Paramecia* to the cc. at that time, or about twelve times the normal dense population.

(*e*) Infusions of 7 liters' volume passed through the same pH cycles, but these cycles were considerably retarded as compared with the 700 cc. infusions.

(*f*) The tendency of infusions to kill the *Paramecium* population was associated with a persistence of the extremely acid condition for a period of several days, as is shown by Plate 2. This prolonged acid condition developed in infusions which were made with too much flour, too much hay, or too much of both ingredients.

(*g*) The radically changing hydrogen ion concentrations which were recorded in successful *Paramecium* cultures were thought to be due at least in part to a changing cycle of bacteria. We plan to try to determine what bacteria are responsible for these changes; the maximum, minimum, and optimum pH for each bacterium, the stages of the infusion in which each bacterium is most numerous; and the pH conditions which the metabolism of each bacterium induces.

(*h*) The extreme concentrations of *Paramecium* which were obtained in cultures which were covered after becoming concentrated by evaporation, where populations of as many as 2000 per cc. were kept for ten days continuously, leads us to question whether excretory matter is as toxic to the organism as has been supposed. It seems probable that the excretory matter becomes broken down and reorganized by bacterial and chemical action before it becomes sufficiently concentrated to injure the animals. It may be possible that in such reactions will be found the explanation of the pH behavior which has been reported.

LITERATURE CITED

Beers, C. Dale, 1927. Factors involved in the Encystment in the Ciliate Didinium nasutum. *Jour. Morph.*, **43**: 499.

Beers, C. Dale, 1927. The Relation between Hydrogen-Ion Concentration and Encystment in Didinium nasutum. *Jour. Morph.*, **44**: 21.

Bodine, Joseph Hall, 1921. Hydrogen-Ion Concentration of Protozoan Cultures. *Biol. Bull.*, **41**: 73.

Child, C. M., and Deviney, E., 1926. Contributions to the Physiology of Paramecium caudatum. *Jour. Exper. Zool.*, **43**: 257.

Crane, M. M., 1921. The Effect of Hydrogen-Ion Concentration on the Toxicity of Alkaloids for Paramecium. *Jour. Pharm. and Exper. Therap.*, **18**: 319.

Darby, Hugh H., 1929. The Effect of the Hydrogen-Ion Concentration on the Sequence of Protozoan Forms. *Arch. f. Prot.*, **65**: 1.

Darby, Hugh H., 1930. The Experimental Production of Life Cycles in Ciliates. *Jour. Exper. Biol.*, **7**: 132.

Fine, M. S., 1912. Chemical Properties of Hay Infusions with Special Reference to the Titratable Acidity and its Relation to the Protozoan Sequence. *Jour. Exper. Zool.*, **12:** 265.

Hargitt, George T., and Fray, W. W., 1917. The Growth of Paramecium in Pure Cultures of Bacteria. *Jour. Exper. Zool.*, **22:** 421.

Johnson, Willis H., 1929. The Reactions of Paramecium to Solutions of Known Hydrogen-Ion Concentration. *Biol. Bull.*, **57:** 199.

Peters, Amos W., 1907a. Methods for the Study of Liquid Culture Media. *Am. Jour. Physiol.*, **17:** 443.

Peters, Amos W., 1907b. Some Chemico-biological Relations in Liquid Culture Media. *Am. Jour. Physiol.*, **18:** 321.

Phillips, Ruth L., 1922. The Growth of Paramecium in Infusions of Known Bacterial Content. *Jour. Exper. Zool.*, **36:** 135.

Pruthi, H. S., 1927. On the Hydrogen-Ion Concentration of Hay Infusions with Specific Reference to its Influence on the Protozoan Sequence. *Brit. Jour. Exper. Biol.*, **4:** 292.